

CLAIMS

- 5 1. A method for replicating poxviruses and recombinants derivatives such as native or recombinant vaccinia virus comprising the steps of inoculating avian embryonic derived stem cells with viral particles and culturing said cells in a basal medium until cells lysis occurs and newly produced viral particles are released in said medium.
- 10 2. A method according to claim 1, wherein inoculation is performed with an m.o.i. (multiplicity of infection) of 0.01 to 0.5.
- 15 3. A method according to one of claims 1 to 2, wherein said vaccinia virus is the modified vaccinia virus such as Modified Vaccinia virus Ankara (MVA) or a recombinant vaccinia virus.
- 20 4. A method according to one of claims 1 to 3 for producing a vaccine against smallpox.
- 25 5. A method according to one of claims 1 to 4, wherein said avian embryonic derived stem cell lines are obtainable by a process consisting of:
 - a) culturing avian embryonic cells in a medium containing all the factors allowing their growth and an inactivated feeder layer,
 - b) passage by modifying the culture medium so as to obtain progressive or total withdrawal of said factors, of the serum and/or of the feeder layer,
 - c) establishing adherent or non adherent cell lines capable of proliferating in a basal medium in the absence of exogenous growth factors, serum and/or inactivated feeder layer.
- 30 6. A method according to claim 5, wherein said avian stem cells obtained in step c) are capable of proliferating for at least 600 days.
7. A method according to one of claims 5 to 6, wherein said avian stem cells are avian embryonic stem cells or avian somatic stem cells.

8. A method according to one of claims 5 to 7, wherein step b) consists in a withdrawal of the components of the medium (growth factors alone or serum alone or growth factors and then serum or alternatively serum and then growth factors).
- 5 9. A method according to one of claims 5 to 7, wherein step b) consists in a progressive or total withdrawal of the feeder layer and then optionally in a withdrawal of the other components of the medium (growth factors and serum).
- 10 10. A method according to one of claims 5 to 9, wherein said avian cell lines are non-adherent stem cells which proliferate in suspension in a medium free of exogenous growth factors.
- 15 11. A method according to one of claims 5 to 9, wherein said avian cell lines are non-adherent stem cells which proliferate in suspension in a medium free of serum (serum-free medium).
12. A method according to one of claims 1 to 9, wherein said avian cell lines are non-adherent stem cells which proliferate in suspension in a medium free of exogenous growth factors and serum.
- 20 13. A method according to one of claims 1 to 13, wherein said avian cell lines have at least one of the following characteristics:
 - a high nucleo-cytoplasmic ratio,
 - an endogenous alkaline phosphatase activity,
 - an endogenous telomerase activity,
 - a reactivity with specific antibodies selected from the group of antibodies SSEA-1 (TEC01), SSEA-3, and EMA-1.
- 25 14. A method according to one of claims 1 to 13, wherein said avian cell lines are cultivated in basal medium, in particular in a medium such as DMEM, GMEM, HamF12 or McCoy supplemented with additives such as nonessential amino acids, vitamins and sodium pyruvate.

15. A method to produce live or attenuated vaccine such as a vaccine against smallpox comprising culturing the adherent or non adherent cell lines established in step c) according to the process defined in one of claims 5 to 14, inoculating said cells with viral particles and culturing said cells in a basal medium as mentioned above until cell lysis occurs and newly produced viral particles are released in said medium.
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16. The use of the non-adherent cells as defined in one of claims 10 to 12 to produce live or attenuated vaccine belonging to the family of orthopoxvirus, in particular vaccinia virus, modified vaccinia virus such as Modified Vaccinia virus Ankara (MVA) and recombinant
10 vaccinia virus.
17. The use according to claim 16 for producing a vaccine against smallpox.
18. The use according to claim 16 for producing a vaccine against cancer.